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SHORTER ARTICLES AND DISCUSSION

MUTATIONS IN ŒNOTHERA BIENNIS L.?

It is evident that the adherents of the mutation theory are sensitive to the doubts freely expressed concerning the status of *Œnothera Lamarckiana,* the behavior of which in throwing off marked variants is cited as the most important evidence for the origin of species by mutations. These doubts are in fact criticisms of the assumption that *Lamarckiana* is representative of a wild species and express the view that this plant is of hybrid origin and that its behavior is of the sort to be expected of a hybrid. Consequently, mutationists are likely to bring forward as rapidly as possible any evidence that may seem to indicate the appearance of clear inheritable variations of a marked character in forms of pure germinal constitution, *i. e.,* in homozygous material.

There are types of *Œnothera* that we have reason to believe are now very pure and have been so for a great many years. Such a form is the *biennis* of the sand dunes of Holland. This species has apparently been established in its habitats in Holland since pre-Linnean times. There has been little opportunity for chance hybridization and its habits of close or self pollination in the bud are greatly in favor of the continuation of its germ plasm in pure lines. Moreover, the type in experimental cultures of De Vries and others has proved to be constant. If then it could be shown that tested strains of this *biennis* are able to produce new forms of specific rank or even marked varieties the mutationists would have much stronger evidence in support of the mutation theory than that based on the behavior of *O. Lamarckiana,* a form unknown as the component of any native flora.

The title of a recent paper, "Mutation bei *Œnothera biennis* L.," by T. J. Stomps,[1] a former student of Professor De Vries, naturally then arouses interest especially since he is working with this same *biennis* of the sand dunes of Holland, a type well known to a number of botanists who are conducting experimental studies on œnotheras. A brief discussion of the claims indicated by the title of this paper, an analysis of the evidence presented and its possible interpretation supplies the chief incentive for this review.

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[1] Stomps, T. J., "Mutation bei *Oenothera biennis* L.," *Biologischen Centralblatt,* XXXII, p. 521, 1912.

The greater part of this paper consists of a discussion of certain criticisms directed against the mutation theory by those who believe that *O. Lamarckiana* is of hybrid origin. Certain objections of Stomps appear to the writer well founded, but we shall not take the space to consider this portion of the paper since the greater interest attaches to the value of the direct evidence offered by him in support of the mutation theory.

When we come to the short account of the experimental work of Stomps we find that the so-called "mutants" were not derived from the pure Dutch *biennis* of the sand dunes but from a cross between this race and a type designated *O. biennis cruciata.* This fact seems to the writer of fundamental importance in judging the conclusions of Stomps. It should be made clear that the form "*O. biennis cruciata*" is recognized in the more recent taxonomic treatments as a true species sharply distinguished from types of *biennis* by its floral characters. Whatever may have been the origin of *O. cruciata* or its possible relationship to *O. biennis,* a cross between these types must certainly be regarded as a cross between two very distinct evolutionary lines and its product a hybrid in which marked modifications of germinal constitution are to be expected.

Œnothera cruciata differs from *O. biennis* most conspicuously in having very narrow linear petals, from 1–3 mm. wide, in sharp contrast to the broad heart-shaped petals characteristic of *biennis. O. cruciata* is found wild in certain regions of New England and New York and is consequently a native American species. Stomps assumes that the *cruciata* in Holland is a mutant from the Dutch *biennis,* but his belief rests upon no direct evidence. *Cruciata* has never appeared in the extensive cultures of the Dutch *biennis* grown by De Vries and Stomps. Neither have we any direct evidence that the American *cruciata* has come from any form of *biennis.* It is true that the species *cruciata* and *biennis* appear to be closely related, but it is equally clear that they constitute very distinct lines each with a long period of evolutionary independence. I can not see the justification for Stomps's attitude when he treats a cross between the *biennis* and *cruciata* of the sand dunes of Holland as though it were the combination of forms within the same species which have similar germinal constitutions.

Stomps lays emphasis on the purity of his material of *biennis* and *cruciata* which had been carried along for several years in pure lines from original wild plants of the sand dunes. He states that the crossing of these two forms is concerned alone with the floral peculiarities of *cruciata*, since in all other characters the two types are the same. It seems to the writer hardly possible that lines so well established as *biennis* and *cruciata* can be absolutely the same in all respects except that of flower form, although this is obviously the most important point of difference. The American forms of *cruciata* are exhibiting among themselves remarkable differences of germinal constitution.

The observations of Stomps are of interest. He obtained in the second generations from crosses between *biennis* and *cruciata* two marked variants. These are called *biennis nanella* and *biennis semi-gigas* because of the similarity to somewhat corresponding variants from *Lamarckiana*. We are not informed as to the proportions in which these new forms arose, a point of importance since we should like to know whether they are very rare, as say 1: 10,000, or more common.

The first variant, *biennis nanella,* appeared in the second generation of the cross *biennis* × *cruciata*. This cross gave an F_1 hybrid with heart-shaped petals as in the mother plant; no statements are made as to the size relations. In the F_2 generation there was a splitting into forms of *biennis* and *cruciata;* we are told nothing of the proportions of these individuals in the cultures or of their range of variation. One of the *biennis*-like forms presented a dwarf habit which distinguished it from the *biennis* parent in much the same way that *nanella* is distinguished from *Lamarckiana*. This plant, *biennis nanella,* differed from *Lamarckiana nanella* by the same characters that distinguish *biennis* from *Lamarckiana*. An important point of resemblance to *Lamarckiana nanella* lay in its sensitiveness to bacteria which Zeylstra discovered within the tissues of this type and showed to be responsible for certain abnormal characters. De Vries has shown that *Lamarckiana nanella* grown in a soil treated with calcium phosphate became healthy and Stomps found that his *biennis nanella* responded in a similar way to this treatment.

The second variant, *biennis semi-gigas,* appeared in a second generation from the reciprocal cross *cruciata* × *biennis*. This cross also gave an F_1 hybrid with flowers of the *biennis* type and in the F_2 generation there was likewise a splitting of the culture into forms of *biennis* and *cruciata.* One of the *biennis*-like forms presented a more vigorous habit and a larger size of buds, flowers and leaves, suggesting the differences between *Lamarckiana* and its derivative *gigas.* The style was longer than in *biennis* and self-pollination, characteristic of *biennis,* was impossible. This plant proved to be almost sterile.

A count of the chromosomes as shown by mitotic figures in meristematic tissue of young buds determined them to be 21 in number. This important fact placed the plant in that group intermediate between the usual types of *Œnothera* with 14 chromosomes and that very rare variant from *Lamarckiana,* called *gigas,* which has 28 chromosomes. It has recently been shown that certain plants that have been mistaken for *gigas* have 21 chromosomes and for these the name *semi-gigas* has been proposed. Consequently Stomps calls the plant from the cross *cruciata* × *biennis* with 21 chromosomes *biennis semi-gigas.*

The observation of this remarkable plant and the determination of its chromosome count is a matter of great interest. The fact that the number of the chromosomes (21) is not twice the number of the parent types (14) shows that the germinal variation did not take place after a normal fertilization, for a doubling of the number of chromosomes in the fertilized egg or embryo would give a plant with 28 chromosomes. It indicates that a gamete produced by one of the plants in the F_1 generation had 14 chromosomes and that this element combining with a normal gamete (7 chromosomes) produced this exceptional plant with 21 chromosomes.

I have suggested[2] a way in which gametes of an *Œnothera* might be formed with 14 chromosomes in place of the normal number. The presence of 28 chromosomes instead of the normal number 14, during a heterotypic mitosis in an *Œnothera* might come about from a somewhat earlier appearance of that premature division of the chromosomes which normally takes place as early as anaphase of this mitosis. Thus a pushing forward of this premature fission of the chromosomes from the anaphase to the metaphase of the heterotypic mitosis would result in the distribution of 14 chromosomes to each pole of the spindle. Another fission introduced before the metaphase of the homotypic mitosis would make possible a group of 4 nuclei at the end of the reduction divisions each with 14 chromosomes. From such nuclei gametes would be formed with 14 chromosomes.

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[2] *Annals of Botany,* Vol. XXV, p. 959, 1911.

The position of Stomps is clear. He believes that the Dutch *biennis* and *cruciata* have identical germinal constitutions except for the factors that determine floral structure and therefore with respect to other characters can be crossed as though they were homozygous. Since the cross gave two marked variants which differed from the parents in other respects than those of floral structure these two plants are mutants. These conclusions are then applied by Stomps to the problem of the status of *Lamarckiana* in the following line of reasoning. Since *biennis* mutates and since *biennis* is probably an older species than *Lamarckiana* it follows that mutations among the œnotheras are older than *Lamarckiana* and that consequently the mutations of this species can not be the result of hybridization.

The line of argument rests primarily on the assumption that *biennis* and *cruciata* have exactly the same germinal constitution except for floral characters. This I can not believe possible considering the long evolution back of the two lines. Why did Stomps find it necessary to cross *biennis* with *cruciata* to obtain his "mutants"? If homozygous in all respects except for flower structure why should not *biennis* alone or *cruciata* alone give the same mutants? From my point of view Stomps really made a cross between two rather closely related species and obtained *first* the segregation of flower types to be expected in the F_2 generation among which from my experience I should expect a wide range of variation, and *second* Stomps obtained two marked variants due to some germinal modification as the result of the cross.

This sort of phenomenon I am obtaining frequently in crosses of my races of American *biennis* and *grandiflora*. The *nanella* condition of dwarfed growth is very common in F_2 generations. And, last summer in an F_3 generation a large plant appeared with leaves so thick and stems, buds and flowers so stocky that I have hardly a doubt but that the cytological examination will show an increase in the number of chromosomes.

In so far as the observations of Stomps bear upon the problem of mutation my interpretation would be exactly the reverse of his. To me they further illustrate the same phenomenon that I am obtaining through my hybrids of *biennis* and *grandiflora*, namely, that behavior by which these hybrids in the F_2 generation throw off variants that in taxonomic practise would be considered new species readily distinguished from the parents of the cross and from the F_1 hybrid. I have this past summer found that F_2 hybrids similar in character to the F_1 will in the F_3 generation repeat the performance of the F_2 and throw off again some of the same marked variants.

It is a satisfaction to know that De Vries and Stomps stand firmly by the original definition of a mutation as a germinal variation (and this means inheritable) from a pure stock, *i. e.*, from homozygous material. This is a valuable concept whether or not mutation proves to be a rare phenomenon. Furthermore, one of the most important lines of experimental study is that which will endeavor to determine with precision the conditions under which true mutations may arise. There has been a loose usage of the term mutation which should it become prevalent will take from the word the significance described above, and reduce it to a meaning no more precise than that of a marked germinal variation from any source. If the word mutation is to be kept in the sense of De Vries it must be reserved for germinal variations from homozygous stock.

BRADLEY MOORE DAVIS

A CONVENIENT MICROSCOPE CASE

A VERY convenient case for holding microscopes, especially for large, beginning courses where two or more students in different sections use the same instrument, is shown in the accompanying photograph.

The case here shown was built to stand in a shallow offset in the laboratory near a door, and fills a small space that would otherwise be wasted. As is seen from the numbers below the sections, it will hold fifty standard microscopes. Each instrument has a number on the base to correspond to the number on its respective section. Across the floor of each section, at the back, is nailed a 2 in. × 2 in. strip of wood to stop the base of the microscope and to serve as a shelf for the extra oculars. Holes of the proper diameter in the shelf would hold these oculars more safely. The doors slide easily on a metal track with ball-bearing wheels and have brass pushes set flush with the surface of the wood. They may be fastened with a catch or with a lock and key.